A distinct human CD4+ T cell subset that secretes CXCL13 in rheumatoid synovitis.

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CITATION:

Kobayashi, Shio ...[et al]. A distinct human CD4+ T cell subset that secretes CXCL13 in rheumatoid synovitis.. Arthritis and rheumatism 2013, 65(12): 3063-3072

ISSUE DATE:

2013-09-10

URL:

http://hdl.handle.net/2433/178763

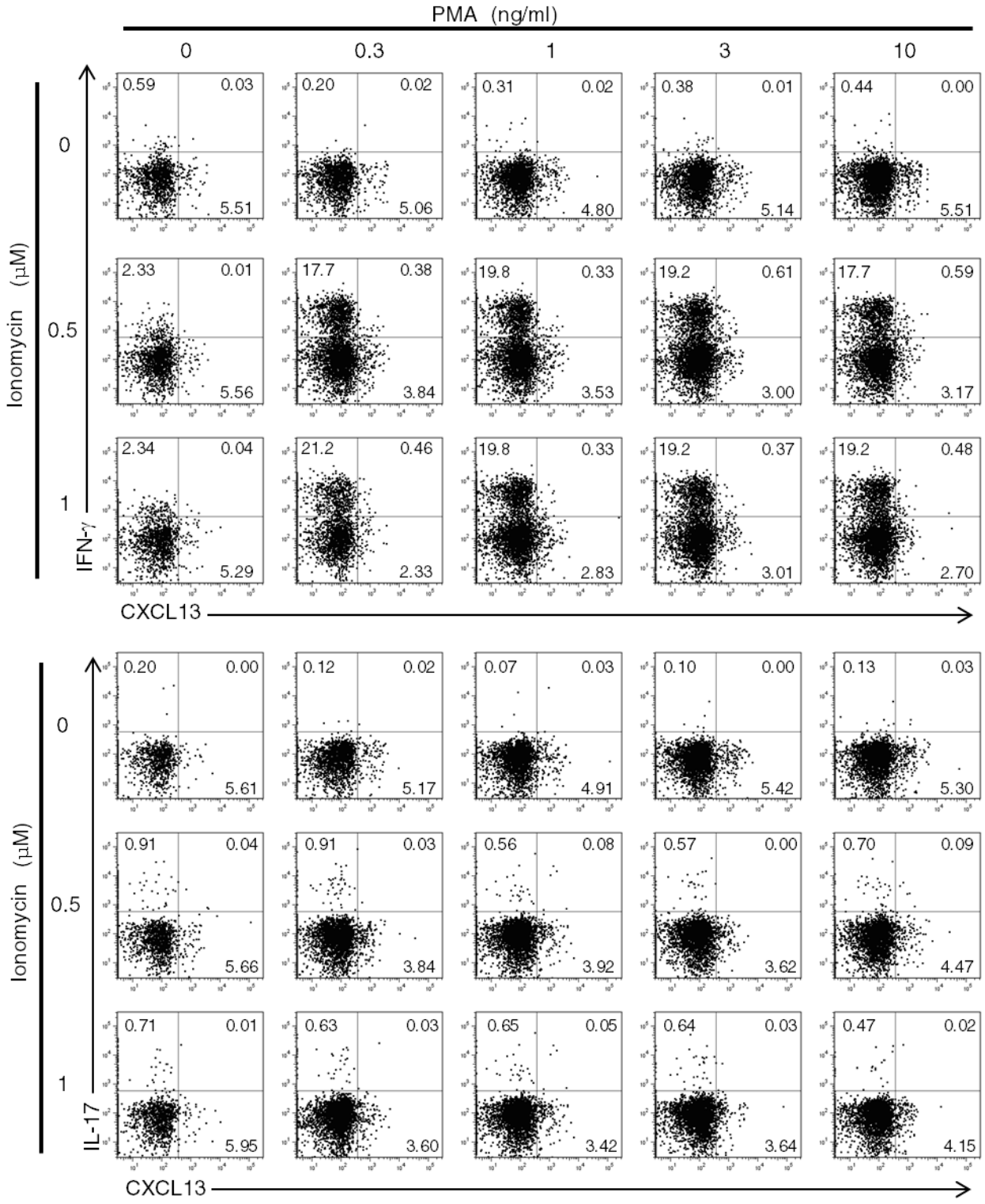

Figure S1. Optimization of the concentration of PMA and ionomycin to detect IFN-γ, IL-17, and CXCL13. $CD4^+$ T cells from rheumatoid synovitis were stimulated with PMA and ionomycin at the indicated concentrations for 5 h and then analyzed by flow cytometry. Numbers in the quadrants represent the percentages of the indicated populations.

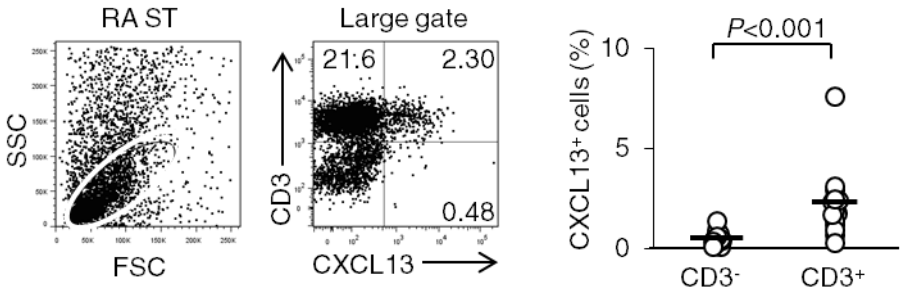

Figure S2. Flow cytometric analysis of synovial tissues including large cells. The CXCL13 production by single cells of RA synovial tissue without PMA–ionomycin stimulation was analyzed (n=14). A representative dot plot gated on cells including larger cells than lymphocytes is shown. Numbers in the quadrants represent the percentages of the indicated populations. Bars represent the mean.

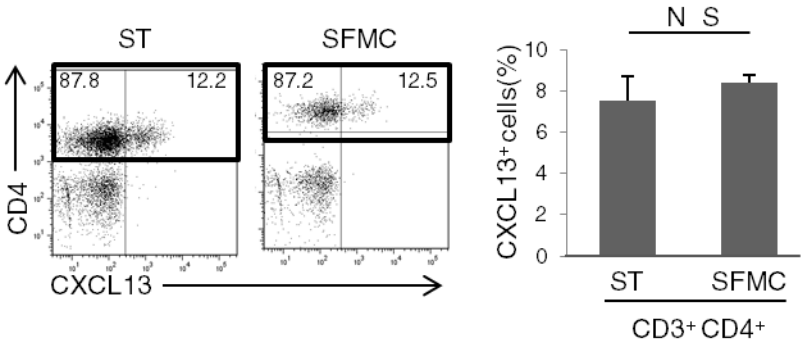

Figure S3. Spontaneous CXCL13 production in synovial tissue (ST) and synovial fluid mononuclear cells (SFMC) of RA. $CD4^+$ T cells from ST or SFMC of RA were analyzed by flow cytometry without PMA–ionomycin stimulation (n=3). Numbers indicate percentage of indicated populations in $CD4^+$ T cells. NS: not significant.

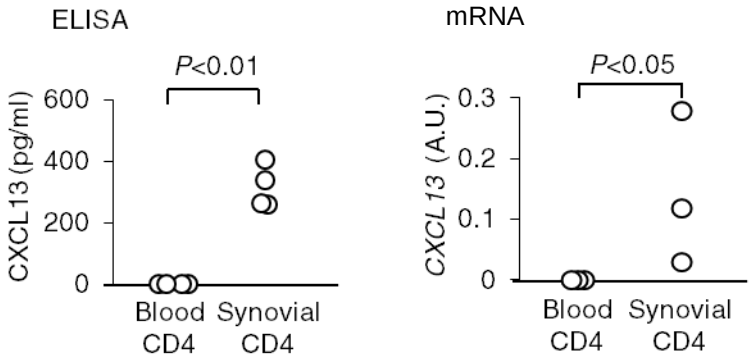

Figure S4. Spontaneous CXCL13 production by blood and synovial CD4+ T cells from RA patients. CD4+ T cells were sorted with magnetic beads and then cultured with complete RPMI 1640 medium at a concentration of 1×10^6 /ml for 36 h at 37°C. The concentration of CXCL13 in the supernatant or synovial fluid was measured by ELISA (left; ST: n=1, SF: n=3). The expression of mRNA in sorted CD4+ T cells was quantified by qPCR using TaqMan Gene Expression Assay (Applied Biosystems); *CXCL13* (Hs00757930_m1) and *GAPDH* (Hs02758991_g1). The expression of *CXCL13* was normalized to the expression of *GAPDH* (right; ST: n=3)

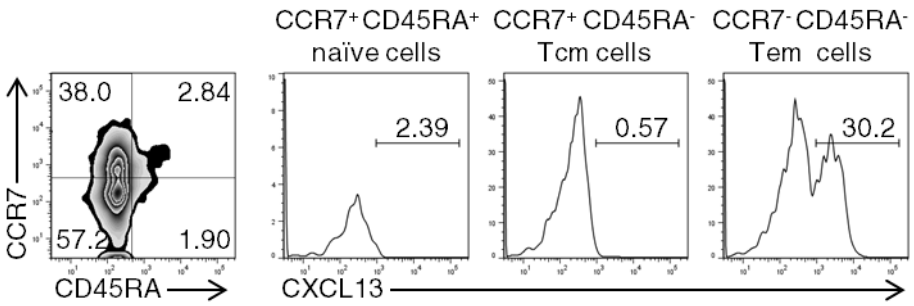

Figure S5. CXCL13+CD4+ T cells of RA synovitis are restricted to effector memory helper T cells. Spontaneous CXCL13 production by CCR7+CD45RA+ naïve cells, CCR7+CD45RA− central memory (Tcm) cells, and CCR7−CD45RA− effector memory (Tem) CD4+ T cells is shown. Numbers in quadrants and histograms show percentages of the indicated populations. Representative data from three independent experiments are shown.

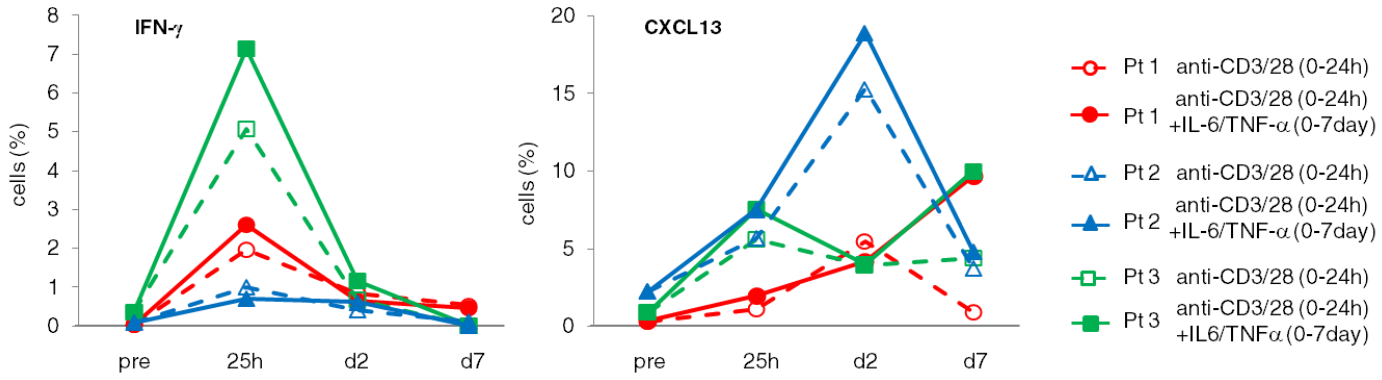

Figure S6. The summaries of the time course for the production of CXCL13 and IFN-γ in three RA patients. Sorted whole CD4+ T cells with low spontaneous production of CXCL13 from RA synovitis were cultures as in Figure 4B. Closed symbols with solid line and open symbols with dashed line indicate cells cultured with and without proinflammatory cytokines, respectively.

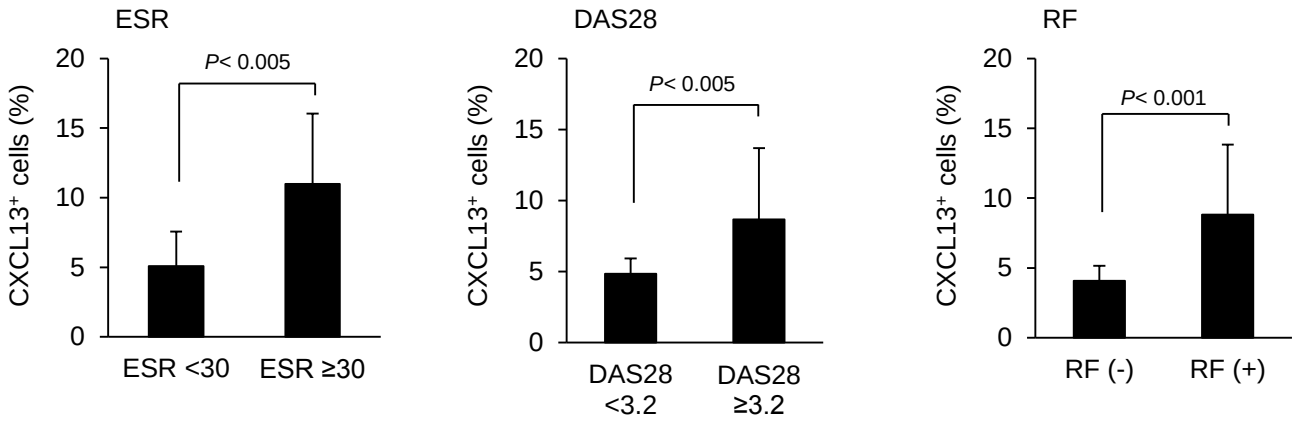

Figure S7. Relevance between RA clinical parameters and iTh13 cells. The frequencies of CXCL13+CD4+ T cells among CD4+ T cells in patient with low ESR (<30; ST: n=10, SF: n=2), high ESR (≥30; ST: n=10, SF: n=2), negative rheumatoid factor (RF) (ST: n=2, SF: n=2), positive RF (ST: n=18, SF: n=2), low disease activity (DAS28<3.2; ST: n=4), and moderate or high disease activity (DAS28≥3.2; ST: n=16, SFMC: n=4) are shown.

Table S1. Clinical details of patients with RA

Number of patients	24
Sex, male/female	7/17
Age	65.1 ± 10.5
Disease duration (y)	13.5 ± 9.24
Positive ACPA, n (%)	22 (91.7)
ESR (mm)	37.9 ± 37.0
C-reactive protein (mg/l)	1.59 ± 1.61
DAS28(ESR)	4.48 ± 1.27
Drug use, n (%)	22 (91.7%)
DMARDs	19 (79.2%)
Steroid	13 (51.2%)
Biologics	3 (12.5%)

SD = standard deviation; ACPA = anti-citrullinated protein antibody; ESR = erythrocyte sedimentation ratio; DAS28 = 28-joint Disease Activity Score; DMARDs = Disease Modifying Anti-rheumatic Drugs.

Table S2. Anti-human antibodies used in this study

Antigen	Manufacturer	Clone	Format	Application
CD3ε	BioLegend	UCTH1	Brilliant Violet	Flow cytometry
CD3ε	BioLegend	UCTH1	PE	Immunohistofluorescence
CD4	BioLegend	RPA-T4	PerCP-Cy5.5	Flow cytometry
CD25	BioLegend	BC96	FITC	Flow cytometry
PD-1	BioLegend	EH12.2.H7	APC-Cy7	Flow cytometry
IL-4	BioLegend	8D4-8	PE	Flow cytometry
IL-17	BioLegend	BL168	PE-Cy7	Flow cytometry
RORγt	BioLegend	AFKJS-9	PE	Flow cytometry
CD19	BD Bioscience	HIB19	FITC	Flow cytometry
CD45RA	BD Bioscience	H100	FITC	Flow cytometry
T-bet	BD Bioscience	O4-46	PerCP-Cy5.5	Flow cytometry
GATA3	BD Bioscience	L50-823	PE-Cy7	Flow cytometry
Bcl-6	BD Bioscience	K112-91	PE	Flow cytometry
IFN-γ	BD Bioscience	B27	FITC	Flow cytometry
CD4	eBioScience	RPA-T4	APC-eFluor780	Flow cytometry
CD20	eBioscience	L26	Alexa488	Immunohistofluorescence
CD69	eBioscience	FN50	PE	Flow cytometry
CCR7	eBioscience	3D12	PE	Flow cytometry
ICOS	eBioscience	ISA-3	PE-Cy7	Flow cytometry
IL-21	eBioscience	3A3-N2	PE	Flow cytometry
Foxp3	eBioscience	236A/E7	PE	Flow cytometry
CXCR5	R&D Systems	51505.111	PE	Flow cytometry
CXCL13	R&D Systems	53610	APC	Flow cytometry / Immunohistofluorescence